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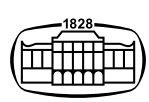

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MEMBER OF WOLTERS KLUWER GROUP

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ABSTRACTS

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New Challenges in Microbiology

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Contents

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Section I. Emerging viral infections

V. 1.

History of Medical Microbiology Institute

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The first institutional microbiology scientific work and teaching activity was established in 1883 by Endre Hőgyes. At that time microbiology and pathophysiology were combined in one subject and it was organized in the General Pathophysiology and Bacteriology Institute. Due to facility improvements in the Institute the number of students increased to 1200. Endre Hőgyes was the head of the Institute for 20 years and during that time scientific and teaching activity was maintained. In 1890 Endre Hőgyes founded the Institut Pasteur in Budapest to continue scientific work that he had started in Paris as a colleague of Louis Pasteur.

In 1948 the Insitute of Bacteriology and Immunity was founded that organized teaching and scientific work of microbiology. In 1950 the head of the Institute was Ferenc Faragó and our Institute was named as Institute of Microbiology.

Adenovirus research team was established in 1956 by István Nász. The scientific acitvity was financially supported by the Hungarian Academy of Sciences that covered positions in the team, participation in congresses and in study tours. Since 1999 our Institute has been named as Insitute of Medical Microbiology

Nowadays, our Institute teaches in three languages (Hungarian, English and German) on three Faculties of Semmelweis University namely, Medicine, Dentistry and Pharmacy. Our Institute takes part also in organizing courses, workshops in different fields of microbiology. Throughout the decades scientific work of our Institute covered bacteriology, virology, microbiome and prions. In 1988 the Microbiology Museum and Archive has been established and maintained by István Nasz, a Full Member of the Hungarian Academy of Sciences. This museum demonstrates all the scientific work including all publications of our Institute from 1948 till today.

V. 2.

Studies on adenoviruses at the Institute of Microbiology

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The Adenovirus Research Group (Institute of Microbiology, Medical University of Budapest) was established by Dr. István Nász (Professor and Head 1974−1994, Member of the Hungarian Academy of Sciences) in the mid 1950s. Three subgroups were formed: 1) Studies on the fine structure of hexon molecules and their comparison by monoclonal antibodies for

gene therapy – Éva Ádám, DPharm, DSc 2) Comparative analysis of type specific DNA – György Berencsi MD, PhD, 3) Studies on the clinical significance of adenoviruses in immunomodulation – Gizella Kulcsár MD, DSc. Major trends in immunomodulatory studies were: Latent adenovirus infection could be established by using minimal inocula or temperature sensitive mutants of HAdV-5 in tissue cultures or mouse lymphocytes. Among several inorganic and organic compounds tested bacterial endotoxin, steroid hormones, prostaglandins and indomethacin could reactivate and augment adenovirus replication via cellular secondary messenger systems. Lymphocytes carrying latent HAdV-1 and HSV-1 are excreted through the gingival sulcus consequently contribute to juvenile periodontitis by decreasing the activity of phagocytes. *In vitro*, their TNF-a production parallel to oncogenic potential was shown to suppress bactericidal capacity of phagocytes. It was shown that circulating and tumor infiltrating lymphocytes frequently express HAdV functions and their immunomodulatory effects may contribute to the progression of malignancies of the male urogenital system. Adenovirus research ceased around the Millenium, but has been re-established at the Institute of Medical Microbiology since 2010s. As HAdVs are important cofactors in AIDS progression and mortality, in the frame of the feline AIDS model AdV serological survey was conducted in Europe: nearly 20% of cats were found seropositive. An infectious agent obtained from the faces of a cat and identified as the first adenovirus isolate in *Felidae* worldwide. Its biological and molecular and characterizations are in progress.

V. 3.

The Adenovirus Research Group of the Hungarian Academy of Sciences, 1995−1998

Orsolya Dobay

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The Semmelweis University − Hungarian Academy of Sciences Adenovirus Research Group was grounded and led by Professor István Nász. When I started working in the Institute of Medical Microbiology in 1995, I became part of this group and had the opportunity to join the ongoing research. In the frame of a veterinary project, we examined the Adenovirus infection in cats. First, we tested 100 cat sera from the Czech Republic, using HAdV-1 hexon antigen. We detected 25% seropositivity, which was higher in females, in very young or very old cats, and cats with respiratory or GI symptoms. Next, 632 cat sera were tested from five different countries. As a result, we could show a strong correlation between cat adenovirus and cat immunodeficiency virus (FIV) infections. Because in all these experiments we used ELISA, in the next study we have optimalised the ELISA procedure using the Taguchi optimalisation method. This sophisticated method can test the effect of different conditions by reducing the interactions between the variables, providing result in one step. Finally, in the last study the adenovirus viropexis was investigated. We could show that the epsilon-coatamer protein plays a crucial role in the endosomal transport, hence enhancing the virus leaving the early endosomes. In this experiment, we used Ad5-Luc3 virus and CAR-transfected CHO cells.

V. 4.

HHV-6 and inhibitory KIR2DL2 NK cell receptor

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Human Herpesvirus 6 (HHV-6) is frequently present in the human population. Infection takes place during the infancy, and thereafter the virus persists in latent form. HHV-6 is mainly lymphotropic, but it can infect also other cell types. Two viral variants have been described. In spite of their similarity, HHV-6A and HHV-6B differ for biological, pathological, functional characteristics and are considered as different viral species. Primary infection by HHV-6B causes Exanthema Subitum, while so far HHV-6A pathogenesis during primary infection still has to be elucidated. Nevertheless, both viruses can reactivate and cause severe diseases, mainly (but not exclusively) in immunocompromised patients. Recently we described association of HHV-6A with autoimmune thyroiditis (Hashimoto's) and female primary idiopathic infertility.

Natural killer (NK) cells, as part of the innate immune system, play a key role in host defense against viral infections. Recent advances have indicated that NK cell activation and function are regulated by the interplay between inhibitory and activating signals. To investigate the possible effect of HHV-6A and HHV-6B infection, as well as of other human herpesviruses, on NK cell response, we will present two models of study: i) infection in Multiple sclerosis patients; ii) infection at the NK/endometrium interface.

We have recently shown that NK cells from a subgroup of Multiple Sclerosis (MS) patients have an impaired response to Human Herpesvirus infection (Rizzo et al. J Neuroimmunol. 2016) and are characterized by the expression of KIR (Killer Ig-like receptors) 2DL2 inhibitory receptor. NK cells were engineered to switch on and off KIR2DL2 expression by demethylation and transfection. The turning on of KIR2DL2 resulted in NK cell inability to control HHV-6A and 6B infection (but also of other human herpesviruses, such as HSV-1 and EBV), with a 3 log increase in viral production. The KIR2DL2 switch off re-established NK cell activation towards herpes-infected cells, to levels detected in parental cells. These results show the potential role of KIR2DL2 in determining susceptibility to herpesviral infection.

We have recently shown the presence of HHV-6A DNA in 43% of endometrial biopsies from primary idiopathic infertile women, but not in fertile women (Marci R et al. PlosOne 2016). The presence of HHV-6A infection seems to affect endometrial specific CD-56brightCD16 − NK cells percentage and activation, with infertile HHV-6A positive women with a decrease in specific endometrial resident NK cells, a different expression of activating and inhibitory receptors and a hampered DNA sensor STING-STAT6 signalling.

For the first time, we show the mechanisms responsible for NK cell activation and function in the control of HHV-6 infection. These data will help the development of antiviral and NK cell-based therapies.

V. 5.

In vitro studies on the immunomodulatory aspects of HHV-6, HHV-7-associated encephalitis

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Roseoloviruses, as human herpesvirus (HHV)-6A, 6B and HHV-7 infect almost all small children worldwide consequently establish lifelong latency in CD4+ immune cells and neuronal cells. Depending on genetic background, geographical regions, immune status or other underlying diseases at any age, these viruses alone or in coinfections with other pathogens can induce different immunological and neurological disorders. Upon primary infection, all three species might elicit sporadic form of encephalitis. HHV-6A can be associated with rhomboencephalitis, HHV-6B with or without exanthema subitum. Delayed HHV-7 infection might induce severe encephalitis with generalized symptoms and lifelong sequelae. The most severe forms of encephalitis develop in immunocompromised patients due to underlying malignancies and immunosuppressive and cytotoxic drug therapy. Encephalitis associated with generalized HHV-6B and CMV reactivation is usually part of generalized symptoms after liver transplantation. Frequently lethal forms of encephalitis arise after bone marrow or haematological stem cell transplantation. Additional risk factors are young age and presence of chromosomally integrated HHV-6A and/or HHV-6B in either donor or recipient, unmatched cord blood cell transplantation and repeated hematopoietic stem cell transplantation. HHV-6 damages the brain stem, hippocampus, limbic tissue. The most severe manifestation is post-transplantation acute limbic encephalitis with extreme high mortality. Beside direct brain cell damages exerted by Roseoloviruses, alterations in the intrathecal cytokine pattern significantly contribute to the disease course. Rapid microbial differential diagnosis, and during disease course and treatment, continuous monitoring of the viral load and cytokines primarily in the cerebrospinal fluid are required. No FDA approved treatment regimen is available. Ganciclovir, valganciclovir, foscarnet or cidofovir therapy or pre-emptive approach have been shown a variable value. Restricted use of immunosuppressive and cytotoxic drugs, avoiding other drugs with known Roseolovirus activating potential would be desirable in an individual basis.

V. 6.

New challenges in CMV infections

Davide Abate

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Human Cytomegalovirus (CMV) represents a prominent cause of fetal infection, causing sensorineural abnormalities, hearing impairment and developmental disorders. At the present several diagnostic methods are available to assess congenital infection during pregnancy,

however a variable fraction (30-2%) of CMV infected pregnant women transmit the virus to the fetus, depending from maternal serostatus at conception. The cell-mediated immune response has been investigated as potential factor involved in the congenital infection. Surprisingly, high levels of maternal T-cell mediated immune response were found to be associated with congenital CMV transmission, especially in presence of maternal low IgG avidity level. Thus, congenital CMV infection may be due to an imbalanced Th1/Th2 response in pregnant women.

V. 7.

The 35 years of HIV/AIDS – What next?

Erwin Tschachler

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It has been 35 years ago that in 1982 the CDC formulated the surveillance definition for the newly occurring acquired immunodeficiency syndrome (AIDS). What followed was a complex success story of biomedical research: already in 1983 was HIV-1 (then HTLV-II/LAV), the infectious agent which causes this disease, discovered and 2 years later tests to detect HIV-1 infections were widely available. Intensive basic research rapidly identified potential drug targets and the first drugs to combat HIV entered clinical trials already in 1986. Ten years later, a highly efficient combination anti-retroviral therapy, which broke the vicious cycle of viral propagation and loss of immune cells, was introduced. These drugs target different steps of the viral life cycle and have transformed HIV-disease from a rapidly progressing fatal illness into a chronic condition. In fact, today both the life expectancy and the quality of life of HIV-infected patients taking antiretroviral therapy is approaching the one of non-infected individuals. The nowadays wide availability of antiretroviral drugs and the introduction of drugs as post exposure prophylaxis have helped to lessen also the spread of the virus. On the downside, despite continuing efforts, the search for effective HIV-1 vaccines have so far not (yet) yielded satisfying results. As to the elimination the virus from infected individuals the fact that HIV-1 target cells that carry mutations in one of its receptors are resistant to infection, strongly hint that the introduction of this receptor variant into hemopoeitic stem cells through genetic engineering might represent a way forward to achieve a cure from HIV-1 infection in the future.

V. 8.

Contribution of advanced molecular virology to the HIV surveillance in Hungary

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In the second decade of the 21st century Hungary is still in a good epidemiological situation concerning HIV infections and AIDS. It is the result of the strict but consistent introduction of nationwide HIV screening in 1985, the good cooperation of clinicians, researchers and government officials, and the early advanced molecular virological research. This latter had been implemented by the author resulting in the first isolation of HIV in Hungary in 1988, virological confirmation of the first AIDS case in the country, and the determination of HIV-1 B subtypes as the major circulating HIV variants in Hungary.

HIV screening in risk groups had been introduced in 1985, when the first infections were confirmed. During the last 32 years altogether 3344 HIV infection (3/100.000) had been reported and the cumulative number of AIDS cases are 892. Distribution of HIV subtypes and genetic resistance (allele frequencies of CCR5-Δ32, CCR2-64I and SDF1-3'A) were determined by molecular virological methods, antiretroviral drug resistance in primary HIV infection and transmission of circulated recombinant forms of HIV (CRF) in migrants were analysed by genotyping (Truegene HIV-1 Genotyping System /Siemens/). New approach in inhibiting HIV infection by modifying SH-groups of CD4 and HIV gp120 *env* using novel thyolated nucleosides was implemented for which HIV viral pseudotypes were developed.

In the last 32 years MSM is still the highest risk group, while HIV infection is low among *i.v*. drug users. Genetic resistance to HIV representing CCR5-Δ32 was found in 12% of general population and 15% in ethnic gypsy minority. Genotyping revealed, that approx. 15% of primary HIV infections are transmitted by drug resistant mutants mainly in MSMs. After 2004, when Hungary joined to the European Union migration increased mainly from SE Asia and Africa, resulting in the appearance of new HIV subtypes and African CRF, such as CRF02_AG (28.5%), CRF06_cpx (17.8%) and CRF11_cpx(3.6%). New pathway of HIV entry mechanism has been revealed, and experimental novel entry inhibitor DS53 developed locally acting on cell surface SH-groups concentrated by cell membrane lipid rafts was effective *in vitro* in 0.75 uM.

Transmission of drug-resistant HIV during primary infection, penetration of African CRFs raise serious clinical and public health consequences. To maintain the recent favourable epidemiological situation screening programs are to be continued, rapid HIV tests had been introduced, HIV genotyping at the time of diagnosis should be the standard of care, prevention policies including PrEP and introduction of novel compounds, among them entry inhibitors in the treatment are necessary.

V. 9.

Vector-borne diseases threatening Europe: dengue, chikungunya, zika

Anna-Bella Failloux

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Some mosquito species have expanded their geographic distribution as a consequence of human activities, both in creating conditions favourable for their proliferation, and introducing means to passively transport them across borders. The Asian tiger mosquito *Aedes albopictus* is emblematic of how passive transport has contributed to both vector invasion, and the subsequent emergence of arboviruses in these regions. First detected in 1979, *Ae. albopictus* is now present in at least 20 European countries and has been incriminated as the main vector of dengue (DENV) and chikungunya (CHIKV) viruses. While eradicated from Europe after World War II, autochthonous cases of dengue have been reported again in Europe since 2010: in France in 2010 and 2014, Croatia in 2010 and Madeira in 2012. After a first outbreak of chikungunya in Italy in 2007, local transmission was confirmed repeatedly in Europe and mainly associated to a CHIKV belonging to the East-Central-South-African genotype. However, the role of *Ae. albopictus* in the transmission of Zika virus (ZIKV) is still unclear as no human cases were reported in Europe despite hundreds of imported cases returning from Zika-epidemic regions of South America and the Caribbean. Nevertheless, as most arboviruses, ZIKV can evolve and become better adapted for a transmission by *Ae. albopictus* like what happened with CHIKV. Based on our vector competence studies, we will discuss on the recent emergence of arboviral diseases in temperate regions.

V. 10.

The Ebola outbreak – is it over?

Bernadett Pályi[1], Nóra Magyar[1] and Zoltán Kis[1,2]

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Ebola virus was discovered in 1976, but until the West-African outbreak in 2013−2016 only sporadic cases were reported and scattered across Africa attributed to the Ebola virus, with maximum a few hundred cases. Ebola Virus Disease (EVD) is a zoonotic disease, and the reservoirs of the virus are different bat species. The deforestation and the growing number of human population increase the likelihood for humans to come into contact with the disease reservoirs facilitating a possible outbreak. The ease of traveling and movement across borders can contribute to the spread of the disease to surrounding countries. Moreover, the poor public health system, shortage of health workforce, financial problems are all important challenges in controlling future Ebola outbreaks in African countries. The virus can persist for a long time especially in male reproductive tract that may enable virus transmission from recovered and non-symptomatic EVD survivors. This new finding is implicating the

initiation of an entirely new way of EBOV transmission. Experimental recombinant Ebola vaccines will be important in the future outbreaks and one dose can generate quick immune response, however it has not been tested in real outbreak situation and it is not clear how long such immunity lasts.

V. 11.

Laboratory diagnosis of Zika virus

Orsolya Nagy

National Public Health Institute, Hungary

The Zika-virus, an arthropod-borne flavivirus, has become a significant public health concern in the last ten years due to its rapid spread to new geographical areas, and association with severe neurological symptoms and intrauterine malformations. Not only its overlapping endemic regions, shared clinical symptoms and common distributing vectors with Chikungunya virus and Dengue virusmake its diagnosis challenging, but the characteristic serological cross reactivity of the *Flavivirus* genus also complicates the evaluation. In Hungary, West-Nile virus and tick-borne encephalitis virus, two important members of the *Flavivirus* genus are widespread. Part of the population is vaccinated against tick-borne encephalitis. Moreover, travellers to tropical and subtropical regions are at risk of getting infected with other flaviviruses such as Dengue or yellow fever virus. Besides describing the complexity of the serological diagnostics of Zika virus, the presentation is aimed at giving an overview of the molecular methods regarding the time of virus shedding in various sample materials. Some interesting case reports will also be highlighted, to give a better understanding of diagnosing Zikavirus infection as a secondary flavivirus infection.

V. 12.

Hepatitis B and C genotypes in Hungary

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Molecular analysis of viral genomes for genotyping is routinely used in the diagnosis of hepatitis B and hepatitis C virus infections. Genotypes are genetic variants of a virus species characterized by the difference in the nucleic acid sequence of their genomes. Genotypes show a specific geographical distribution and they may also differ in their virological features, clinical outcome of the infection and response to antiviral therapy. To date, 10 hepatitis B virus (HBV) genotypes have been described. They show more than 8% genetic divergence and are further classified into sub-genotypes differing by 4−8% of the genome. In Hungary HBV genotypes A and D are the most common, but occasionally genotypes B, C and G have

also been detected. Infection with genotypes A and D result in chronicity at a higher rate than B and C. Genotype D shows the worst response to interferon therapy. Hepatitis C virus (HCV) strains are classified into 7 genotypes and a large number of subtypes. About 90% of Hungarian HCV carriers are infected with genotype 1 viruses, and the majority of them are subtype 1b. Genotype 3 is more common in intravenous drug users than the general population. Genotypes 2 and 4 also occur, but are rare. Genotype 1 and especially subtype 1b has been the most difficult to treat using interferon-based therapy, but with the latest interferon-free regimens sustained viral response is over 90%.

V. 13.

The experience with the treatment of Hepatitis C infection

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Approximately 0.7% of the population of Hungary (70,000 patients) can be infected with hepatitis C virus (HCV). The majority of them is unaware of their condition.

The pegylated interferon + ribavirin dual treatment that has been used between 2003 and 2012 on patients infected with HCV genotype 1 (G1) could have cured 40-45% of naïve patients, and 5-21% of patients who received prior, failed treatments.

Addition of I. generation DAA (direct acting antivirals) – protease inhibitors (BOC/TVR) to dual therapy with PEG IFN-alfa with RBV, yielded SVR rates 63-75%; 59-66% respectively.

From 2014, other direct acting antivirals are registered for the treatment of chronic hepatitis C in different combinations, including short duration (8-12 weeks) interferon-free regimens, with a potential efficacy over 90%.

Due to budget limitations therapy is covered only for a proportion of pts by the Hungarian National Health Insurance Fund. Approved treatments are restricted to the most cost-effective combinations based on the cost/SVR value in different patient categories with recommendation of the Hungarian Hepatitis Therapeutic Committee.

We have a nationwide uniform database, Hepatitis Registry (HepReg). In the HepReg are about 1200 pts on the waiting list.

All treatments that are covered from a pre-defined budget by the National Health Insurance Fund are to be centrally approved. These treatments are restricted to the most cost-effective combinations based on the cost per sustained viral response value in different patient categories with consensus between professional organizations, Insurance Agency and patient organizations.

In Hungary GT 1 is the most frequent HCV GT (about 95%), and the incidence of HCV, subtype 1b is dominant.

We have collected data from Hungarian Hepatitis Registry since 2013, who suffered chronic hepatitis C GT 1b. The patients with fibrosis stadium were more than 75% − F4 (by fibroscan screening).

We achieved the good SVR results from IFN based therapy, because these patients have F1 fibrosis stadium, mostly IVDU patients from prison. The efficacy of triple therapy (PEG IFN + RBV + Boceprevir or Telaprevir) was not superior, than IFN-based therapy.

The IFN-free therapy was introduced in Hungary in the year 2015. The efficacy was excellent (> 96% SVR), taken into account that fibrosis stadium treated patients were mostly F4-F3.

Cirrhotic pts who achieve an SVR should remain under surveillance for HCC every 6 months by ultrasound (like pts with advanced fibrosis, METAVIR score F3), and for oesophageal varices by endoscopy if varices were present at pre-treatment endoscopy.

The presence of cofactors for liver diseases, such as history of alcohol drinking, obesity and/or type 2 DM, may determine that additional assessment are necessary.

Long-term post-SVR follow-up studies showed that, the risk of developing HCC remains in pts with cirrhosis who eliminated HCV.

Screening for HCV infection is presently based on the detection of anti-HCV antibodies.

If anti-HCV antibodies are detected, HCV RNA should be determined to identify pts with on-going infection, and make a decision to treat this patient and achieve a SVR.

V. 14.

The evolution of molecular diagnostic in HPV

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Cervical cancer preventive screening as in the past as it is today one of the most successful areas of diagnostic, medical developments. From early cytology concepts to the improved, standardized, present-day form of the cytology and more recently HPV testing and modern triage markers, we can withstand the continuous improvement of the cervical cancer managing tools. At present these technologies not only co-exist but augment each other. This process of technological accretion has involved the widespread adoption of novel automated technologies: liquid-based cytology (LBC), robotized HPV detection platforms and automated slide readers. This presentation explores the consequences of this development for today diagnostic and clinical managment and provides insight into future developments.

Section II.
The challenges of the multidrug resistant bacteria and new therapeutic approaches

B. 15.

Global trends in the antimicrobial resistance

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In recent years resistance levels in important Gram-positive pathogens such as *Staphylococcus aureus, Streptococcus pneumoniae*, and Enterococcus species have only increased to a modest degree and in some cases even decreased. This has been paralleled by development of several novel drugs targeting gram positive pathogens. In gram-negative pathogens a steady increase in resistance levels has been observed over the last decade, resulting in a very concerning situation with regards to cephalosporin resistance in *Escherichia coli* and both-cephalosporin and carbapenem resistance in *Klebsiella pneumoniae*. Moreover, the last years an increase in colistin resistance has also been observed, particularly among carbapenem-resistant *K. pneumoniae*. *The Acinetobacterbaumannii*-group is increasingly often observed in blood cultures and a high proportion of the isolates are resistant to carbapenems. During the same time resistance in *Pseudomonas aeruginosa* has not increased to the same degree as for other Gram-negative pathogens. The presentation discusses overall trends and relation to clonal expansion of some epidemic clones carrying important resistance determinants, as well as the importance of asymptomatic carriage as a source for the emergence of resistant strains in severe infections. Finally, emerging resistance to recently introduced antimicrobial agents is also discussed.

B. 16.

Multidrug resistant Gram-negative pathogens in Hungary

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Multidrug resistant (MDR) Gram-negative pathogens are an ongoing challenge in Hungary. During the decades the ease as these pathogens have disseminated in the country and contributed to the increased number of infections is remarkable. The first reports regarding MDR Gram-negatives in Hungary date back to 1998 and 1999 when the first SHV-2 and SHV-5 extended-spectrum beta-lactamase (ESBL)-producing clinical isolates of Enterobacteriaceae

appeared. In 2005 widespread dissemination of CTX-M-15 type ESBL-producing *Klebsiella pneumoniae* was related to high-risk clones namely, ST11, ST15 and ST147. These clones developed resistance against fluoroquinolones, aminoglycosides and sulfonamid.

Emergence of carbapenem resistance among *Pseudomonas aeruginosa* and other Gram-negatives represents alert in numerous hospital wards in Hungary. First reports of VIM-4 producing *P. aeruginosa* were published in 2004 and later on it was detected all over the country. *Aeromonas hydrophila*, *Klebisella* spp. and *Enterobacter cloacae* carrying integron-borne *bla*VIM-4 were also detected in Budapest. Hence, reports of NDM, OXA-48-like and KPC in our country still VIM is the dominant carbapenemase.

Recently, appearance of acquired colistin resistance has been observed. An outbreak of KPC-2 producing *K. pneumoniae* ST258 in North-east Hungary was the first detection of acquired colistin resistance, however sporadic cases of colistin resistant *Escherichia coli*, *Enterobacter asburiae, Pseudomonas spp.,* and *Acinetobacter baumanii* have been reported to date.

B. 17.

Multi-drug resistant Gram-positive bacteria in Hungary

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Infections caused by multidrug-resistant Gram-positive bacteria represent a major public health burden, not just in terms of morbidity and mortality, but also in terms of increased cost on patient management and implementation of infection control measures. Among these *Staphylococcus aureus* and *Enterococcus* spp. are established pathogens in the hospital environment, and their frequent multidrug resistance complicates or even makes the therapy impossible.

Although, European mean percentage for methicillin resistance in *S. aureus* (MRSA) decreased significantly between 2013 and 2016 (from 18.1% to 13.7%) based on the EARS-Net data, in Hungary the rate of methicillin resistance among invasive *S. aureus* isolates increased (25.6% in 2016) with alternating favourable and unfavourable changes in the last 10 years. In this period the population structure significantly changed among epidemic MRSA strains. While New York/Japan (ST5-MRSA-II) and Southern German (ST228-MRSA-I) clones were predominant in the early 2000s these were replaced by EMRSA-15 (ST22-MRSA-IV) by end of the 2000s but the proportion of ST5-MRSA-II increased again in the last 5 years. The relevance of MRSA outside of hospitals (CA-MRSA, LA-MRSA) has also increased in the last 10 years, moreover the first case of *mec*C-positive MRSA has been described in 2016.

The European mean percentage for vancomycin resistance in *Enterococcus faecium* was 11.8% in 2016 and the trend did not change significantly during the period 2012−2016. In Hungary the vancomycin resistance was below the European mean in 2012 (7.1% vs 9.0%) but was double in 2016 (22.4% vs 11.8%). The molecular epidemiological results suggest that countrywide spread of hospital adapted, *vanA*-positive *E. faecium* lineages (e.g.

BAPS2.1a and BAPS3.3a − formerly called CC17) harbouring pRUM-like plasmids with unique Tn1546::IS1251 transposon variant could have caused the sharp increase in vancomycin resistance after 2012. Furthermore, since 2015 a *vanA*-positive *E. faecalis* CC28 strain showing low level β-lactam resistance and harbouring unusual *vanA*-coding- plasmid disseminates in Hungary. Thus, efforts to optimize the use of antimicrobials against Gram-positive bacteria and to recognize emerging mechanisms of resistance have to be one of the major public health and clinical priorities.

B. 18.

Global trends in the seroprevalence in *Streptococus pneumoniae*

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Streptococcus pneumoniae remains a major cause of infectious disease, especially among the very young (children <5 years of age) and the elderly (>60 years of age). Apart from causing diseases like otitis media, pneumonia and meningitis, pneumococci colonize the nasopharynx, with the highest level of carriage reported from pre-school age children.

Pneumococcal conjugate vaccines (PCVs) have been developed to reduce the burden of disease among children and, through mucosal immunity, also reduce the carriage of serotypes included in the vaccine. So far, three different PCVs have been used for the vaccination of children in many countries worldwide: PCV7, containing antigens for 7 pneumococcal serotypes, PCV10 (10 serotypes) and PCV13 (13 serotypes).

In countries that vaccinated children under the age of two years this has had a profound effect on the incidence of pneumococcal disease both among vaccinated children as well as among non-vaccinated children and adults (herd protection). Incidence of vaccine serotypes have been strongly reduced, but non-vaccine serotypes have increased in prevalence (replacement). In most countries incidence of invasive pneumococcal disease among children has reduced. Among adults, herd protection has changed the serotype distribution, with vaccine serotypes disappearing, but the overall burden of disease seems mostly unaffected.

B. 19.

Pneumococci carried by healthy children in Hungary, 2009−2015

Orsolya Dobay

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Streptococcus pneumoniae is one of the leading human pathogens, and it is still the cause of death of half million children <5 years old worldwide. The infection often derives from symptomless carriers, usually from small children. To prevent pneumococcal infections, polysac-

charide and conjugate vaccines (PCVs) were developed, both based on the most important antigen of the bacterium: the polysaccharide capsule. Although these vaccines contain only a limited number of serotypes out of the total of 94 (PCV 7, 10 or 13), they were proven to be remarkably successful in decreasing pneumococcal infections, and also influenced carriage. On the other hand, pneumococci responded rapidly to the vaccine pressure and a large scale serotype arrangement was observed all over the world.

Vaccination with PCV-7 basically started in 2009 in Hungary, and PCV-13 became an obligatory vaccine in 2014. We have followed the serotype changes among the carried pneumococci between 2009 and 2015. While in the first few years the old serotypes dominated, later these mostly vanished, and previously rare types emerged. Especially the multi-resistant serotype 19A (which is present only in PCV-13) emerged worryingly, but later it was also suppressed successfully. Due to the dynamic changes in serotypes, pneumococci should be monitored continuously and new vaccines should be developed.

B. 20.

Antibiotic therapy in the age of multiresistant bugs − a clinician's approach

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The rapid spread of the multiresistant bacteria poses a difficult challenge for the medical community in the treatment of severe infections. Though several new antibiotics will be launched in the near future, their availability will not solve the problem of bacterial resistance. The basic principles of the treatment of severe infections are well established: adequate antibiotic treatment (combination) should be introduced within 3 hours from the recognition of the infections. The use of antibiotic combinations in empiric therapy in cases presumably due to multiresistant bugs is clearly indicated together with deescalation strategy. However, the superior clinical efficacy of combinations in targeted therapy is controversial in infections caused by carbapenemase producer Enterobacteriacea, *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* despite of the plethora of positive in vitro results. It seems, that the use of antibiotics according to their pharmacodynamic characteristics may improve their clinical efficacy. The introduction of individualized therapy based on the regular monitoring of antibiotic serum concentrations might be an important step forward.

B. 21.

Dissimilar fitness associated with resistance to fluoroquinolones influences clonal dynamics of various multiresistant bacteria

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Authors' group previously demonstrated that diverse fitness cost associated with resistance to fluoroquinolones has substantially contributed to the dissemination of the major international clones of methicillin-resistant *Staphylococcus aureus* (MRSA) and multiresistant *Klebsiella pneumoniae*. Since the major clone isolates are multiresistant their spread resulted in elevations also in the rates for non-fluoroquinolone antibiotics (like β-lactams, aminoglycosides, macrolides). The findings were subsequently confirmed by other laboratories who also showed that a similar mechanism was responsible for the dissemination of the major clones of two additional pathogens (multiresistant *E. coli* and *Clostridium difficile*). Authors review the related literature and demonstrate that a more judicious use of fluoroquinolone type antibiotics should result in an amelioration of the general antibiotic resistance situation.

B. 22.

FDA Pathways to Antibiotic Approval

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The process of transforming a molecule to a medical therapeutic in order to secure United States marketing authorization is well-established, but can often seem confusing. This confusion can be even more pronounced with antibiotic regulatory submissions, due to the growing number of options available to drug sponsors from the US FDA. The emergence of these options is due in part to heightened fear over antibiotic resistance and the mounting worry that novel treatments will be unavailable to patients suffering from resistant infections. Such concerns have resulted in new legislation, such as the FDA Safety and Innovation Act (FDASIA) and the 21st Century Cures Act (Cures Act). In this session, we will discuss the history of the FDA's drug-review process, the various pathways available to secure antibiotic approval, and a handful of recent approvals to illustrate some of these more creative routes to approval.

B. 23.

Mechanistic studies on short proline-rich antimicrobial peptides

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Proline-rich antimicrobial peptides (PrAMPs) are a large diverse family expressed in many insects (~20 residues) and mammals (~60 residues) except humans. Thus, they represent interesting lead structures for further pharmaceutical developments to support the human innate immune system. Indeed, several research groups have proven the high efficacies of optimized PrAMPs in different murine infection models. Their bactericidal mechanism was partially elucidated by identifying chaperone DnaK as binding partner and transporter SbmA as part of the bacterial uptake system. We could show that slight structural changes of PrAMPs allow them to use an additional transporter system reducing the risk of bacterial resistances, which simultaneously increased also the antibacterial activity. The uptake of short PrAMPs appears to be irreversible and most PrAMPs are either not degraded by cellular enzymes or the degradation products bind the target as well as the full-length peptide. In the cells, PrAMPs target chaperone DnaK disturbing most likely proper protein folding or the ribosome. We could map the binding site of oncocin to the peptide exit tunnel of the 70S ribosome with dissociation constants in the low nanomolar range, which was confirmed for further PrAMPs afterwards. The large binding site of oncocin overlaps with the binding sites of different small molecule antibiotics. Expectedly, these PrAMPs inhibit protein translation efficiently in vitro and in vivo. Remarkably, apidaecin and a few other PrAMPs bind equally well to the ribosome without inhibiting protein translation in vitro. In vivo, apidaecin-type PrAMPs disturb the assembly of the large 50S subunit of the bacterial 70S ribosome forming dead-end intermediates that cannot assemble to functional ribosomes. Inhibition of protein translation is a lethal effect that bacteria cannot escape, which explains also their long lasting post-antibiotic effect.

B. 24.

Are the peptid antibiotics the future?

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Compared to small molecule drugs, peptide therapeutics to human targets offer increased specificity and selectivity and lower toxicity. For better or worse, the in vivo efficacy of antimicrobial peptides include a number of bacterial targets and host defense mechanisms. On one hand, this multimodal activity allows biological actions in a wider playing field and lower risk of resistance induction. On the other, the in vitro individual activity contributions may not be enough to justify drug development in a regulatory setting when measured rela-

tive to benchmark figures. Having said this, the specific biodistribution pattern and preferred sites of action of peptides may allow monotherapy in specific cases such as skin conditions or complicated urinary tract infections. When used in combination with legacy antimicrobials, antimicrobial peptides may specifically target resistance mechanisms or proteinaceous toxin production in very low doses, giving second lives to existing small molecule antimicrobials and host defense peptide therapies alike.

B. 25.

Experiences with A3-APO antibacterial peptide

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Antimicrobial peptides (AMPs) were first thought to fight microorganisms by disintegrating bacterial membranes and later by inhibiting bacteria-specific intracellular processes. However, ever increasing evidences indicate that AMPs accumulate around and modulate the immune system both systemically and in cutaneous and mucosal surfaces where injuries and infections occur.

The proline-arginine-rich antibacterial peptide dimer A3-APO was designed based on a statistical analysis of peptide and protein sequences derived from natural insect products. The *in vitro* activities of A3-APO are due to multiple biochemical effects, including bacterial membrane disruption, inactivating DnaK, the 70-kDa bacterial heat shock protein. DnaK deactivation was shown to inhibit proper folding of bacterial proteins, including resistance enzymes and proteinaceous toxins. Our *in vitro* experiences confirmed that A3-APO inhibits growing of bacteria including moreover multidrug resistant strains at low concentrations. It also inhibits *in vitro* bacterial toxin production, and in combination recovers the efficiency of small molecular antibiotics against resistant strains.

A3-APO was exceptionally active in a series of systemic and local animal models of bacterial infection. ESBL producing *Escherichia coli*, multiresistant *Acinetobacter baumannii*, KPC producing *Klebsiella pneumoniae*, Methicillin resistant *Staphylococcus aureus*, and anaerobe *Propionibacter acnes* were used as infective agents. The readout of efficacy was improvement of survival and reduction of blood or tissue bacterial load. The peptide was active *in vivo* even if it completely failed to kill the pathogenic strains *in vitro*. Multiple lines of evidence suggested that A3-APO fights infections, at least partially, by preventing inflammation at the site of infection.

Section III.
The importance of the human microbiome

M. 26.

The bioinformatical analysis of the microbiome

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The human intestinal microbiome contains over 100 trillion cells, thousands of species and a commensurate huge diversity of genes and functions. This environment, coupled to the enormous data generated by modern NGS sequencing, represents unique challenges when it comes to analysis. The Biomics Unit at the Institut Pasteur has developed at toolbox of pipelines to address this problem. SHAMAN (http://shaman.c3bi.pasteur.fr/) is a SHiny application for Metagenomic ANalysis including the normalization and the differential analysis based on the best current statistical models of metagenomic data. SHAMAN is coupled MASQUE which handles 16S/18S/ITS sequence analysis and to and multiple visualization and MBMA which is used for identification of reads from shotgun metagenomics. A further tool was recently developed which permits the reconstruct of the genome of microorganisms from shotgun metagenomic data. This tool is especially important in strain identification and functional prediction along with the statistical analysis in clinical studies. Here we present the current state-of-the-art techniques for metagenomic studies. Examples and pitfalls in the sequencing and analysis of metagenomic data will be presented.

M. 27.

The effect of the antibiotics on the microbiome

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The discovery and use of antibiotics have changed modern medicine. However, the excessive consumption of antibiotics creates many threats, because antibiotic therapy can affect not only the target pathogen but also the commensal flora of human host. The antimicrobial treatment is known to cause short-term changes in the composition of the normal human microbiota, but also long-term consequences have been shown. The effect of antibiotic-induced microbiota alteration increases the susceptibility to intestinal infections, which can stem from newly acquired pathogens or from the sudden overgrowth and pathogenic behavior of opportunistic organisms already present in the microbiota. In particular, antibiotic associated diarrheas due to *Clostridium difficile* are frequent occurrence.

The use of antibiotics selects antibiotic resistant strains of bacteria, but not only in those which the antibiotic is directed towards but also among the normal microbiota. The dysbiosis

brought about by antibiotics bear the added disadvantage of enriching the microbiota in resistant organisms. The human gut microbiota has been established as a significant reservoir of antibiotic resistance genes (ARG). ARGs are detected not just in adults, but also in children and in infants. Furthermore ARGs can be stably maintained in the human gut microbiome in the absence of direct antibiotic selection. Human gut is not just the site of accumulation of ARGs, but also the environment where these genes can spread among different species.

Although, the consequences of long-term persistence of antibiotic resistance in the human gut are currently unknown, there are high risks that this could lead to increased prevalence of antibiotic resistance and reduce the possibility of successful future antibiotic treatments.

Antibiotic induced long-term alteration can also affect the basic immune homeostasis relating to autoimmune diseases, the regulation of host metabolism relating to energy homeostasis and adiposity.

M. 28.

Exercise and the microbiome

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The diversity of the microbiome is associated with cardiovascular fitness. The microbiome of individuals with higher levels of VO2max tend to produce greater amounts of butyrate, which is an important short-chain fatty acid to suppress inflammation. It has also been observed that exercise induces a more diverse microbiome. This is an important observation even though exercise has not been thoroughly linked to gut integrity. Exercise normally reduces the risk of gastrointestinal cancer, reflux, and incidence of ulcers, fatty liver, irritable bowel syndrome, and diverticulitis. In addition, exercise in older animals has been demonstrated to reduce expression of inflammatory mediators and apoptotic markers in intestinal lymphocytes, suggesting a protective role of exercise in intestinal health. Regular exercise can even reduce the progress of Alzheimer Diseases in transgenic mice, by down-regulating pro-inflammatory bacteria in the microbiome, like *Bacteroides thetaiotamicron* and *Lactobacillus johnsonii* which level correlates well with the number and area of amyloid plaques. In addition, *L. johnsonii* produces hydrogen peroxide, therefore the suppression of this bacteria might be linked to the mechanism by which exercise attenuates colon cancer.

M. 29.

The role of the microbiome in the intensive care

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Critical illness is associated with loss of "health promoting" commensal microbes and overgrowth of pathogenic bacteria referred to as dysbiosis. Many intensive care interventions may further disrupt the homeostasis of the microbiome. Dysbiosis may increase susceptibil-

ity to sepsis and organ failure, thus leading to increased morbidity and mortality. Characterizing, understanding and influencing changes in intensive care unit (ICU) patients' microbiome may help to improve patient care. While it is well-known that some interventions (eg. pro-, symbiotics, faecal microbiota transplant, selective bowel decontamination) aim to directly alter the composition of the microbiome, we are rarely aware of the impact of everyday intensive care on the holobiont. The lecture overviews the evidence based risks and benefits of these medical interventions with direct or indirect influence on the microbiome. Current evidences describing the effect of these interventions on the microbiome give an explanation while adhering to the guidelines of antimicrobial therapy, stress ulcer prophylaxis, clinical nutrition, mechanical ventilation have significant impact on the outcome. Deliberate implementation of ICU interventions with a microbiome conscious approach improves patient outcome. Further studies are needed to investigate the effect of medical care on the holobiont.

M. 30.

Characteristics of microbiom in paediatric- and adult patients with Crohn's disease

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Inflammatory bowel disease (IBD) − including Crohn's disease (CD) and Ulcerative colitis (UC) − is a complex disease in which genetic and environmental (mainly the diet) circuits establish and contribute to disease pathogenesis. Recent large-scale genome-wide association studies link IBD to host-microbe pathways central to sensing and signalling and mucosa-initiated effector responses. However, patterns of gut microbiome dysbiosis in paediatric- and adult IBD patients are inconsistent among published studies. Analysing samples from multiple gastrointestinal locations collected prior to treatment in new-onset cases, a characteristic pattern of microbiome in paediatric CD was established. Namely, an increased abundance in bacteria which include Enterobacteriaceae, Pasteurellacaea, Veillonellaceae, and Fusobacteriaceae, and decreased abundance in Erysipelotrichales, Bacteroidales, and Clostridiales, correlates strongly with disease status were described. In addition, microbiome comparison between CD patients with and without antibiotic exposure indicates that antibiotic use amplifies the microbial dysbiosis associated with CD. Comparing the microbial patterns between intestinal segments indicates that at this early stage of CD assessing the mucosa-associated microbiome may offer unique potential for convenient and early diagnosis of CD. Based on the recent Paediatric CD Guideline (2014), Exclusive enteral nutrition (EEN) is a first-line therapy in paediatric CD thought to induce remission through changes in the gut microbiome. With microbiome assessment largely focused on microbial taxonomy and diversity, it remains unclear to what extent EEN induces functional changes that thereby contribute to its therapeutic effect. Recent paper published by Dunn et al. faecal samples were collected from paediatric CD patients prior to and after EEN treatment. Metagenomic data were obtained via next-generation sequencing pathway abundance was compared between CD patients and

controls, and between CD patients that sustained remission and those that did not sustain remission (NSR). Eight pathways differed significantly between baseline CD patients and controls. In addition, examination of these eight pathways showed sustained remission patients had greater similarity to controls than non-sustained remission patients in all cases. Knowing the several differences between paediatric- and adult IBD it is not surprising that microbiom of those population is different.

In summary, relative abundance of Bacteroidetes is increased and Firmicutes decreased in CD compared with healthy controls. Enterobacteriaceae, specifically *Eschericia coli*, is enriched. *Faecalibacterium prausnitzii* is found at lower abundance in CD and in those with postoperative recurrence.

M. 31.

Molecular signalling mechanisms underlying the stability of microbial communities

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Members of multispecies bacterial communities (MBC) communicate and cooperate via diffusible molecules that they release into the environment, while also compete for space and resources. Examples of such communities include microbial mats of the oceans, the gut microbiota of animals and insects as well as the microbial communities of the soil. The best studied molecular mechanism is quorum sensing that helps bacteria to orchestrate gene expression programs underlying collective behaviour. In spite of the constant competition, MBCs can be remarkably stable over time and resilient to changes in the environment. *In silico* models suggest that common signals released into the environment may help selected bacterial species cluster at precise locations and that sharing of molecular signals and cooperation factors can stabilize this coexistence. In contrast, unilateral eavesdropping on signals produced by a potentially invading species may protect a community by keeping invaders away from limited resources. Shared bacterial signals, such as those found in quorum sensing systems, may thus play a key role in fine tuning competition and cooperation within multibacterial communities, including the formation self-defense and regulatory equilibria that in turn seem essential for maintaining the stability and/or metabolic repertoire of a community. In particular, horizontal gene transfer seems to enhance the resilience of microbiomes against invading bacteria. Differential responses to related signals on the other hand seem to help communities to keep slower growing microbial species within the community thereby the metabolic repertoire will be maintained.

POSTER SECTION

P. 1.

Clinical and microbiological characteristics of adult invasive *Haemophilus influenzae* infections: results of a 12-year single center experience

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Introduction
Since the introduction of the childhood vaccine for *Haemophilus influenzae* serotype b, the burden of invasive *H. influenzae* disease shifted to older adults and non-vaccine preventable strains. However, only few observational studies were conducted among adults. Our aim was to describe the clinical and microbiological characteristics of adult invasive *H. influenzae* disease in a Hungarian cohort of patients.

Methods
We conducted a single-center retrospective case series study of adult patients treated at our institution between 2004 and 2016 with microbiologically proven invasive *H. influenzae* disease. The hospital database was used by searching ICD-10 codes consistent with *H. influenzae* disease. All consecutive cases with a compatible clinical course and ≥1 microbiological sample positive for *H. influenzae* from a sterile site were included. Hospital and available surveillance data from the National Center for Epidemiology concerning molecular typing were reviewed. Microbiological identification and antibiotic susceptibility testing was done at our institution. Main outcomes were clinical cure, complication rate, empirical antimicrobial therapy, antimicrobial susceptibility and serotype distribution. A post-discharge follow-up was carried out by database search until last documented hospital visits.

Results
Altogether 32 patients (median age 56.3±18.2 years; 59.3% females) were included, average disease incidence corresponded to 0.1 cases / 100.00 inhabitants / year. Patients mostly had diabetes mellitus, cardiovascular and malignant comorbidities (median Charlson score 4), a previously unknown illness was diagnosed in 6.3%. According to SIRS based definitions, 56.3% of cases had severe sepsis or septic shock, 50% were accompanied by bacteraemia. Identified sources were mostly pneumonia and meningitis. Non-typable *H. influenzae* strains

were most prevalent (76.5%), followed by *H. influenzae* serotype f (11.8%). Ampicillin resistance was detected in 15.4% and from these, 11.5% were also resistant to amoxicillin/clavulanic acid. 15.4% of isolates were resistant to trimethoprim/sulfamethoxazole, 3.8% to macrolides. No resistance was found for 3rd generation cephalosporins, carbepenems, tetracyclines or quinolons. 93.8% of patients survived with ceftriaxone or levofloxacine as empirical therapy (78.1%), complications were detected in 34.4%. 22 patients were followed (median follow-up 17.0±26.8 months): 18.2% died, 13.6% had other severe infections, 13.6% had comorbidity deterioration.

Conclusions
In our cohort of adult patients, most invasive *H. influenzae* disease were caused by non-typable and serotype f *H. influenzae* strains with low prevalence for ampicillin resistance. High clinical cure and disease complication rates were observed.

P. 2.

Clinical and microbiological characteristics and outcomes of community acquired sepsis: results of a single center, 1-year retrospective observational cohort study

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Introduction
Community acquired sepsis (CAS) is a life-threatening systemic reaction to infection starting within ≤72 hours after hospital admittance. However, clinical and microbiological data concerning CAS, especially among Hungarian patients are sparse.

Methods
Our aim was to retrospectively analyse the prospectively collected clinical and microbiological data of CAS among a cohort of consecutive adult patients admitted to our 140-bed tertiary referral center during a 1-year study period of 2016. Patients satisfying pre-defined criteria of possible healthcare associations were excluded. Sepsis, severe sepsis and septic shock were defined according to ACCP/SCCM adult criteria. Primary outcomes were in-hospital all-cause mortality and ICU admittance, secondary outcomes were length-of-stay (LOS) measures, rates of bacteraemia, species and antimicrobial susceptibility of causative organisms according to available microbiological data.

Results

206 patients were included in the study (mean age 58.4±20.4 years, 56.7% female), median Charlson score was 4. Calculated incidence of CAS was 285 / 10.000 hospital admittances per year. 66/206 (32.0%) cases were severe sepsis, 59/206 (28.6%) were septic shock. Frequent signs of onset were fever (184/206, 89.3), two-thirds of shock cases presented with tachypnea (61.%, $p < 0.01$ vs. sepsis) and altered mental status (67.8%, $p < 0.01$ vs. sepsis). Prevalent infective sources were genitourinary (51/206, 24.8%), abdominal (51/206, 24.8%) and cardiopulmonary (28/206, 13.6%). Septic shock mainly arose from abdominal sources (39.0%, $p < 0.01$ vs. sepsis), whereas sepsis was caused by urinary infection (32.1%, $p < 0.01$ vs. septic shock). Causative organisms were predominantly *E. coli* (56/216, 25.9%), *S. aureus* (14/216, 6.5%) and *S. pneumoniae* (15/216, 6.9%). In quarter of cases, the causative organism(s) could not be identified (54/216, 25.0%). Community acquired MRSA and ESBL producing Gram negatives as causatives were found with low prevalence (<2% of all cases). Bacteraemia was proven in half of cases (104/206, 50.5%). Empirical antibiotic therapy was mainly ceftriaxone (107/206, 52.1%), escalation was needed in some (34/206, 16.5%). In-hospital mortality was 29/206 (14.1%) for the entire cohort and was highest among patients with shock (0% vs. 6.1% vs 42.1%, $p < 0.001$). ICU admittance was 40/206 (23.8%), and half of shock cases needed ICU support (6.2% vs. 24.2% vs. 55.9%, $p < 0.001$). Mean LOS was 13.1 ± 13,7 days, mean ICU LOS was 12,0 ± 12,2 days.

Conclusions

In our pilot study among adult patients, CAS possessed significant disease burden and mortality in severe cases. A dominant proportion of causative pathogens were susceptible, therefore a carbapenem-sparing approach might be favourable among these patients. More prospective studies are warranted.

P. 3.

Species/serotype distribution and antibiotic susceptibility of Salmonella and Campylobacter sp. isolated from human disease: results of a 1-year observational, microbiological study from Hungary

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Introduction

Acute bacterial foodborne diseases possess high morbidity in Europe. During recent years in Hungary, the incidence of campylobacteriosis exceeded the incidence of salmonellosis. Further data concerning species/serotype distribution and antibiotic susceptibility of enteropathogenic bacteria cultured from human stool samples are needed. Our aim was to analyze available microbiological data of consecutive adult patients presenting with clinical symptoms consistent with acute diarrhoea to assess these parameters.

Methods

A secondary descriptive analysis of data extracted from a prospectively collected electronic database between January 1 and December 31, 2016 was carried out. All in- and outpatients were screened for eligibility by searching for appropriate admittance and discharge codes according to ICD-10 codes. Microbiological data of patients were included if acute diarrhoea was proven and stool samples were sent for microbiological culturing. Acute diarrhoea was defined as ≥3, Bristol 5-7 stools per ≤24 hours for 2–14 consecutive days. At our institution, fresh stool samples are screened for *Salmonella, Campylobacter, Shigella* and *Yersinia* sp. by routine culturing methods. Species identification is done by MALDI-TOF MS, antibiotic susceptibility testings and interpretation are executed according to current EUCAST guidelines.

Results

Out of 4865 stool samples, 812 (16.7%) were culture positive. 516 (10.6%) samples grew *Campylobacter* sp. Among them, 413 (80.0%) were *C. jejuni*, 58 (11.2%) were *C. coli*, and 45 (8.7%) were non-identifiable. 296 (6.1%) samples grew *Salmonella* sp., 154 (52.0%) were *S.* Enteritidis, 63 (21.3%) were *S.* non-Enteritidis and 79 (26.7%) were non-identifiable. Antibiotic susceptibility testing was done for 447 (86.6%) *Campylobacter* and 249 (84.1%) *Salmonella* isolates. Most *Campylobater* strains were resistant to 1 or 2 types of tested antibiotics (42.1% and 41.6%), *Salmonella* isolates were dominantly susceptible to all examined antibiotics (67.5%). Among *Campylobacter* isolates, resistance to ciprofloxacin was exceptionally

high (83.4%), but virtually no erythromycin resistance was observed (0.6%). All *Salmonella* strains were susceptible to ceftriaxone, rates of ampicillin and ciprofloxacin resistance were similar (20.5% and 12.4%). However, *S.* non-Enteritidis isolates carried significantly higher rates of resistance to ampicillin (35.7% vs. 8.0%), trimethoprim/sulfamethoxazole (5.4% vs. 0%) and ciprofloxacin (17.9% vs. 8.0%) compared to strains of *S.* Enteritidis.

Conclusions

The positive detection rate of routine stool culturing was relatively low. The high rate of quinolone resistance among *Camplyobacter* isolates might be due to veterinary overuse of antibiotics. Community acquired acute diarrhoea should probably not be treated with fluoroquinolones empirically. Ceftriaxone may be a first drug for invasive Salmonella infections.